PERCUTANEOUS PENETRATION ENHANCEMENT OF PROPRANOLOL HYDROCHLORIDE FROM HPMC-BASED HYDROETHANOLIC GELS CONTAINING TERPENES

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Abstract

The aim of the present study was to investigate the enhancing effect of five terpenes (menthol, camphor, eucalyptol, thymol and α-bisabolol) at 5% w/w concentration on the *in vitro* percutaneous penetration of propranolol hydrochloride (PRHCl) from hydroxypropylmethylcellulose (HPMC) gels containing ethanol 60% w/w. Permeation experiments were performed on excised pig ear skin. Permeation (flux, permeability coefficient, lag time) and release (release rate, diffusion coefficient) parameters of PRHCl in the gels with and without terpene enhancers were calculated. The results showed that each of the studied terpenes increased significantly the transdermal permeation of PRHCl through pig ear skin compared to control. Eucalyptol was the most effective accelerant, producing the highest enhancement ratio of flux (ER_{flux} = 12.74) and the lowest lag time (1.97 h). Menthol and α-bisabolol showed mild enhancing activity, PRHCl having a rate of permeation 8.98- and 6.74-fold higher than that of control and a lag time of 4.12 and 2 h respectively. The enhancing effect of thymol on PRHCl skin permeation was lower than that of menthol, although the drug showed comparable lag time values in the presence of both terpenes. In the presence of camphor, PRHCl exhibited the lowest flux and a lag time value similar to that of thymol containing gel. The *in vitro* PRHCl permeation through pig ear skin was best described by the zero-order model (in the case of the control gel and that

with camphor) and by the Korsmeyer-Pepas model (in the case of all the other studied formulations). The results suggest the potential use of HPMC-based hydroethanolic gels containing 5% eucalyptol or α-bisabolol as vehicles for topical delivery of PRHCl.

Rezumat

În această lucrare s-a studiat efectul a cinci terpene (mentol, camfor, eucalyptol, timol şi α-bisabolol), utilizate ca promotori de absorbţie în concentraţie de 5% (m/m), asupra penetraţiei percutanate *in vitro* a clorhidratului de propranolol (PRHCl) din geluri pe bază de hidroxipropilmetilceluloză (HPMC) conţinând 60% (m/m) etanol. În experimentele de permeaţie s-a folosit piele excizată de pe ureche de porc. S-au calculat parametrii specifici permeaţiei (flux, coeficient de permeabilitate, timp de latenţă) şi eliberării (viteza de eliberare, coeficient de difuzie) PRHCl în gelurile conţinând sau nu terpenă ca promotor de absorbţie. Rezultatele au arătat că fiecare dintre terpenele studiate au mărit semnificativ permeaţia transdermică a PRHCl prin pielea de porc comparativ cu formularea control. Eucaliptolul a fost cel mai eficient accelerant, producând cel mai mare factor de creştere al fluxului (ER_{flux} = 12,74) şi cel mai scăzut timp de latenţă(1,97 h). Mentolul şi α-bisabololul ca promotori de absorbţie, au prezentat o activitate uşor mai scăzută, PRHCl prezentând o viteză de permeaţie de 8,98 şi respectiv 6,74 ori mai mare decât cea a formulării de control, precum şi un timp de latenţă de 4,12 şi respectiv 2 h. Creşterea permeaţiei prin piele a PRHCl produsă de timol a fost mai scăzută decât în cazul mentolului, deşi substanţa medicamentoasă a prezentat un timp de latenţă comparabil în prezenţa ambelor terpene. În prezenţa camforului, PRHCl a prezentat cel mai redus flux şi timp de latenţă similar celui obţinut în cazul timolului. Cedarea *in vitro* a PRHCl prin piele prelevată de pe urechea de porc a fost cel mai bine descrisă de modelul de ordinul zero (în cazul gelului de control şi al celui cu camfor) şi de modelul Korsmeyer-Pepas (în cazul celorlalte geluri studiate). Rezultatele sugerează posibilitatea utilizării gelurilor hidroetanolice pe bază de HPMC conţinând 5% eucalyptol sau α-bisabolol ca vehicule pentru eliberarea topică a PRHCl.

Keywords: propranolol hydrochloride (PRHCl), hydroethanolic gel, percutaneous penetration, terpenes.

Introduction

Propranolol hydrochloride (PRHCl), known as (2RS)-1-[(1-methylethyl)amino]-3-(naphthalene)-1-yloxy)propan-2-ol hydrochloride, is a non-selective beta-blocker widely used in the treatment of hypertension, cardiac arrhythmias, *angor pectoris* and prophylactic in the recovery from myocardial infarction [27]. Moreover, in the last five years, oral [14, 20, 28] and topical [4, 12] propranolol has been reported to be an effective treatment for infantile haemangioma. After oral administration, PRHCl is rapidly and almost completely (90-100%) absorbed from the gastrointestinal tract (GIT), but has a short half–life (3-6 hours in human) and a relatively low systemic bioavailability (of only 25-30%) due to the extensive hepatic first pass metabolism, which requires an increased administration frequency [19, 22]. These properties of PRHCl make it an ideal candidate for

percutaneous application, explaining the growing interest in developing dermal and transdermal delivery systems of this drug [9, 10].

In the last two decades, gels in general and hydrogels in particular, have been intensively studied as pharmaceutical semisolid dosage forms able to achieve efficient drug delivery after oral, rectal, vaginal, ocular, cutaneous and subcutaneous administration. Thus, hydrogels have become widely used in the biomedical and pharmaceutical domains as drug delivery systems and biomedical devices because of their biocompatibility, network structure, and molecular stability of the incorporated bioactive compound [18]. Hydrogels can be prepared from a wide variety of materials, such as semisynthetic polymers (i.e cellulose derivatives), which have experienced greater growth and development in terms of their practical applications [1, 3, 8, 18]. Due to their high water content, hydrogels can dissolve hydrosoluble drugs, resulting transparent aqueous gels.

On the other hand, the percutaneous penetration of PRHCl is poor because it is a polar, hydrosoluble cationic molecule. Therefore, in order to improve the PRHCl penetration through the skin, several approaches have been investigated [16, 17]. A widely used chemical technique is the coadministration of penetration enhancers, which increase temporarily the permeability of the *stratum corneum*. Several classes of penetration enhancers have been extensively investigated. Although many chemical compounds such as surfactants, alcohols, dimethylsulphoxyde, N-methylpyrolidone, faty acids/esters, urea and its derivatives revealed satisfactory performance in enhancing percutaneous penetration of drugs, the skin irritation caused by some of them restricted their use in topical formulations [13].

During the past twenty years, investigations have been focussed on the screening of safe and effective penetration enhancers from both natural occurring and synthetic chemicals. Terpenes isolated from natural sources (i.e. natural volatile oils) and synthetic terpenoids have gained great interest as they exhibited low skin irritation potential and high ability to enhance drug permeability at low concentrations (1-5%). Further, some terpenes extracted from plants are included in the list of Generally Recognized as Safe Compounds issued by the FDA (Food and Drug Administration, USA). Therefore, terpenes are considered to be less toxic than surfactants and other classes of penetration enhancers [13, 21].

For terpenes and terpenoids as penetration enhancers, two main mechanisms of action have been proposed: (1) they alter the natural barrier of the *stratum corneum*, by interacting with its lipids and/or keratin and (2) they increase the solubility of the drug into *stratum corneum* lipids.

Furthermore, when terpenes or terpenoids are associated with cosolvents like propyleneglycol or ethanol, currently used in topical formulations, synergistic effects have been observed [21].

Various terpenes and terpenoids such as d-limonene, geraniol, menthol, citronellal, camphor, eucalyptol (1,8-cineol), α-bisabolol, borneol, nerolidol, have been used to increase the percutaneous penetration of different drugs including 5-fluouracil, indomethacin, caffeine, hydrocortisone, antipirine and diclofenac sodium [13, 21]. Also, menthol and related terpenes have been tested as enhancers of percutaneous penetration of propranolol hydrochloride from hydrocolloid solution and hydrogel-patch formulation [6, 7, 11].

The aim of this research was the comparative evaluation of the enhancing effect of four monoterpenes namely menthol, camphor, eucalyptol and thymol and of a sesquiterpene (α-bisabolol) on the *in vitro* percutaneous penetration of PRHCl from HPMC-based hydroethanolic gels through excised pig ear skin. The above mentioned terpenes were selected from different chemical classes of aromatic and aliphatic alcohols, ketones and ethers.

Materials and Methods

Materials

Propranolol hydrochloride was a kind gift offered by S.C. Sintofarm S.A (Bucharest, Romania). Menthol, camphor and thymol were purchased from Merck KGaA (Germany), whereas eucalyptol and α-bisabolol were obtained from Sigma-Aldrich Chemie GmbH (Germany). The chemical structures of the terpenes used in this study are depicted in Figure 1.

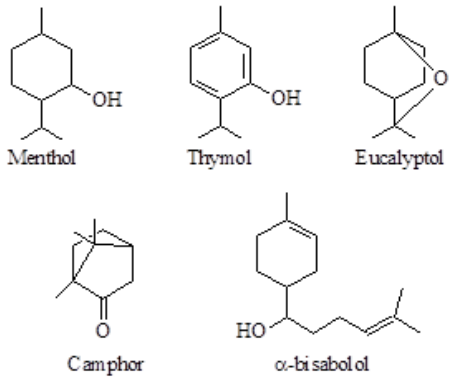


Figure 1.
The structural formulas of terpenes used in the present study

Hydroxypropylmethylcellulose (Methocel K4M, Colorcon L.t.d., UK) was received as gift sample. Ethanol (96%) was purchased from Chimopar S.A. (Romania). Tuffryn HT synthetic hydrophilic membranes of polysulfone (0.45 μm, 25 mm) were supplied by Pall Coorporation (USA). Double distilled water was used throughout the study. All chemicals and reagents were of pharmaceutical or analytical grade and were used without further purification.

Methods

Preparation of HPMC-based hydroethanolic gels of PRHCl

The composition of *PRHCl* hydroethanolic gel formulations is shown in Table I. Formulations were prepared by incorporation of ethanolic solution of *PRHCl* or *PRHCl* and a terpene into a hydrogel base of HPMC (3% and 2% respectively). Briefly, *PRHCl* and the terpenes were dissolved in ethanol 96% (m/V). For obtaining the hydrogel base of HPMC, the polymer was dissolved in water following the "hot/cold" technique [29]. Afterwards, the ethanolic solution of the drug, with or without the terpene was added to the HPMC hydrogel base under gentle continuous stirring.

In all formulations, the drug and the terpenes were dissolved in the hydroethanolic gel and their theoretical concentration were 3% (w/w) and 5% (w/w) respectively. All the samples were stored at 5°C for 24 hours before testing.

Table I

Composition of *PRHCl* hydroethanolic gels

Components	Weight (%) and formulation codes					
	PRHCl	*PRHCl-MTL*	*PRHCl-CMF*	*PRHCl-TML*	*PRHCl-ECL*	*PRHCl-BBL*
Propranolol hydrochloride	3.0	3.0	3.0	3.0	3.0	3.0
HPMC	3.0	2.0	2.0	2.0	2.0	2.0
Ethanol 96% (m/V)	60.0	60.0	60.0	60.0	60.0	60.0
L-menthol	-	5.0	-	-	-	-
D-camphor	-	-	5.0	-	-	-
Thymol	-	-	-	5.0	-	-
Eucalyptol	-	-	-	-	5.0	-
α-bisabolol	-	-	-	-	-	5.0
Distilled water	34.0	30.0	30.0	30.0	30.0	30.0

Drug – excipients compatibility studies

The UV spectrophotometric studies were performed for the PRHCl hydroethanolic gels with or without terpene (menthol, camphor, eucalyptol, thymol or α-bisabolol) and for terpene hydroethanolic gels with no drug. The gel samples were spreaded as a very thin layer between 2 rectangular plates which were fixed in an appropriate support. The gel samples spectra in UV domain (240-340 nm) were recorded using an UV-VIS spectrophotometer (Spectronic Unicam, UV 300 model, USA), and then the correlation for 200 values of wavelength between absorbances from spectra of PRHCl hydroethanolic gels with or without terpenes (menthol, camphor, eucalyptol or α-bisabolol) was performed. Unlike the aforementioned terpenes, thymol has a significant absorption band in the approached spectral domain. Therefore, in this case, a bi-linear correlation for the spectral data obtained for PRHCl hydroethanolic gel with thymol, on one side, and for the individual spectral data obtained for PRHCl gel and thymol gel, on the other side, was performed. The extent to which the mixture spectrum correlates with a proper linear combination of individual spectra was expressed by the multiple correlation coefficients (R^2) between the mixture spectrum experimentally obtained and the mixture spectrum recalculated by linear expression.

Determination of PRHCl content in hydroethanolic gels

To determine the drug content, about 1 g of hydroethanolic gel was weighed in a 100 mL volumetric flask, and dissolved in methanol. 1 mL of this solution was diluted appropriately and the PRHCl content was analysed spectrophotometrically (T70+ UV-VIS spectrophotometer, PG Instruments, U.K.), at 290 nm, against a blank prepared in the same way, using a hydroethanolic gel without the drug. Each assay was performed in triplicate.

In vitro skin permeation studies

Preparation of the skin

In vitro permeation studies were carried out using dermatomed pig ear skin with a surface area of 1.767 cm^2. The skin was excised from 4-month-old domestic pig female or male ears, obtained from a local slaughterhouse. The pig ears were cleaned up with tap water immediately after excision. The outer region of the ears was clipped of bristles and then the skin was dermatomed to a thickness of around 500 μm. The dermatomed skin samples were immediately used for the permeation experiments or stored at –20°C for a maximum period of 2 months. Before use, the dermatomed pig skin was removed from the freezer and allowed to thaw at room temperature. The integrity of the skin was examined, the thickness of

each sheet was measured with a micrometer and then squares of 2 to 2.2 cm^2 were cut from the skin sheet.

In vitro permeation study

The evaluation of *in vitro* PRHCl permeation was performed on Franz diffusion cells (Microette-Hanson system, 57-6AS9 model, Hanson, USA) with an effective diffusional area of 1.767 cm^2 and 6.5 mL of receptor cell capacity. Sink conditions were achieved in the receiver compartment with 6.5 mL freshly prepared phosphate buffer saline (pH 7.4) as receptor fluid. The skin pieces were mounted carefully on the Franz diffusion cells, between the donor and receptor compartments, with *stratum corneum* facing donor chamber. After that, the skin pieces mounted in the cells were allowed to rest in contact with phosphate buffer saline (pH 7.4) 1 h prior the application of the formulations. Approximately 300 mg of the tested formulation was placed into each donor compartment. The receiver fluid was constantly stirred at 600 rpm and the diffusion cells were maintained at 32±1°C throughout the experiment. 0.5 mL sample of the receptor medium were withdrawn at predetermined intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22 and 24 h) and replaced with an equal volume of fresh receiver medium to maintain a constant volume. PRHCl content was measured using an UV spectrophotometric method, at 290 nm. The assay was linear in the PRHCl concentration range of 10-130 μg/mL ($y = 0.097x$, $R^2 = 0.9998$). Three replicates of each experiment were performed.

Data analysis of in vitro drug release studies

The cumulative amount of PRHCl which permeated through the membrane (μg/cm^2) was plotted as a function of time (t, h). The permeation rate of drug at steady-state (flux, J_s, μg/cm^2/h) and the lag time (t_L, h) were calculated from the slope and the x intercept of the linear portion of the plots of cumulative amount of drug permeated *versus* time in steady state conditions, respectively. The permeability coefficient (K_p, cm/h) was calculated by dividing the flux with initial concentration of drug in donor compartment. The enhancement ratios of flux (ER_{flux}) were calculated from the following equation:

$$ER = \frac{J_{ss}\, with\ percutaneous\ enhancer}{J_{ss}\, without\ percutaneous\ enhancer}$$

The release rate (k) values were calculated using the pseudo steady-state slopes from plots of cumulative amount of PRHCl which permeated through membrane (μg/cm^2) vs square root of time. Diffusion coefficient (D) values were calculated from the release rate values.

In order to investigate the release kinetics of the PRHCl from HPMC-based hydroethanolic gels, the data obtained from *in vitro* drug release studies were fitted into various mathematical models, as follows:

- zero order model: $M_t = M_0 + K_0t$, where M_t is the amount of drug dissolved in time t, M_0 is the initial amount of drug in the solution (it is usually zero), K_0 is the zero order release constant expressed in units of concentration/time, and t is the time;
- first order model: $logC = logC_0 - K_1t/2.303$, where C_0 is the initial concentration of drug, K_1 is the first order rate constant, and t is the time;
- Higuchi model: $M = K_Ht^{1/2}$, where M is the amount of drug released in time t and K_H is the Higuchi release constant;
- Korsmeyer-Peppas model: $M_t / M_\infty = K_Pt^n$, where M_t / M_∞ represents the fraction of drug released at time t, K_P is the Korsmeyer-Peppas release rate constant, and n is the diffusion coefficient. In this case, the first 60% drug release data were incorporated.

The following plots were obtained: cumulative percentage drug released *vs.* time (zero-order kinetics), log cumulative percentage of drug remaining *vs*. time (first-order kinetics), cumulative percentage drug released *vs.* square root of time (Higuchi model) and log cumulative percentage drug release *vs*. log time (Korsmeyer-Peppas model).

Statistical data analysis

The statistical analysis was performed using Statistica 7.0 software. Data were shown as mean ± standard deviation (SD) and were considered statistically significant at $p < 0.05$.

Results and Discussion

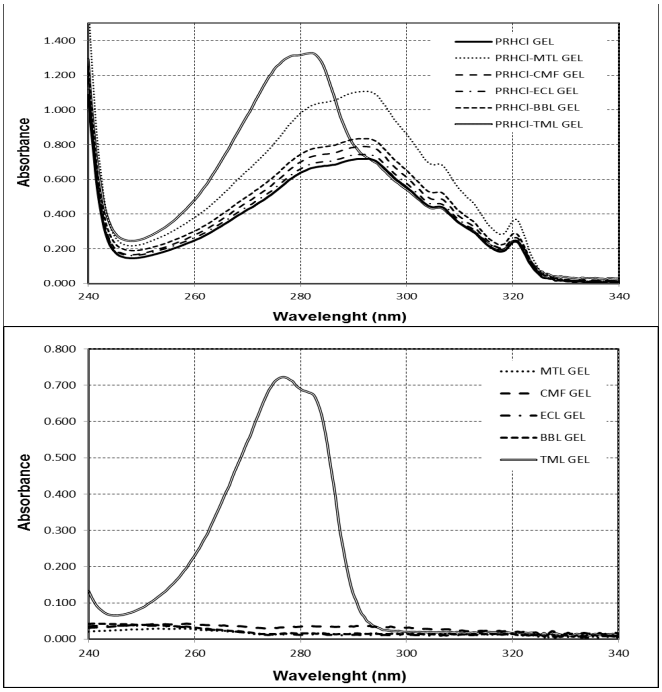
Formulation of HPMC-based hydroethanolic gels of PRHCl

In the process of formulating the PRHCl hydroethanolic gels, it was observed that for the same gelling polymer concentration (3%, w/w), the formulations containing terpenes were slightly more viscous and consistent than the formulation with no terpenes. As the rheological properties of gels have notable influence on drug release, in the terpene containing formulations the HPMC concentration was reduced to 2% (w/w). In the formulations containing terpenes, ethanol was used in concentration of 60% (w/w) as cosolvent for terpenes, since these highly lipophilic compounds, especially α-bisabolol, have poor water solubility when ethanol amounts are less than 60% [7]. On the other hand, it is known that ethanol, widely employed as chemical enhancer [26], and terpenoids have synergistic effects

(acting as penetration enhancers) when they are used in combination [24]. Taking all these into consideration, for an accurate assessment of the enhancing effect of terpenes on the *in vitro* percutaneous penetration of PRHCl from studied formulations, ethanol was also included in the composition of PRHCl gel without terpenes, which was considered as "control" formulation. In this PRHCl hydroethanolic gel, ethanol acted only as penetration enhancer, as the drug is very slightly soluble in water and consequently does not require the presence of a cosolvent to remain dissolved in the hydrogel.

Drug – excipients compatibility studies

In order to test the existence of possible interactions between PRHCl and different terpenes (menthol, camphor, eucalyptol, thymol and α-bisabolol), absorption spectra of propranolol in the medicated gels with or without terpenes and of terpenes in the plain gels were recorded and compared in the UV domain (240-340 nm). The results are shown in Figure 2.



a)

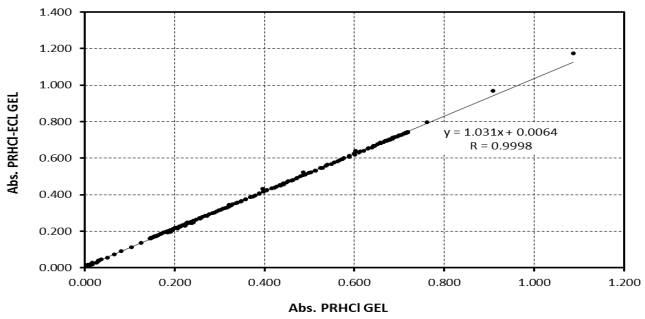
b)

Figure 2.

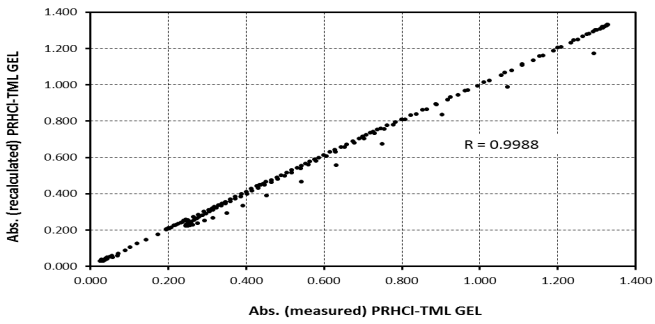
UV spectra of different PRHCl hydroethanolic gels with or without terpenes (a) and of plain hydroethanolic gels with terpenes (b)

The UV spectrum of PRHCl hydroethanolic gel (without terpene) showed three peaks at 290 nm, 305 nm and 320 nm (Figure 2a), while those

of plain gels containing menthol, camphor, eucalyptol and α-bisabolol, did not present any peak (Figure 2b). The obtained value of the correlation coefficient (R^2) is an indication of possible interactions between the drug and auxiliary substances in the studied gels [23]. A notable interaction would be reflected in the dramatic decrease of the value of correlation coefficient (R^2), compared with the theoretical value. In the present study, the absorbance values of spectra recorded for PRHCl hydroethanolic gels containing 5% menthol, camphor, eucalyptol or α-bisabolol, showed a linear relationship with the absorbance values of PRHCl gel spectrum, linearity indicated by the correlation coefficient values very closed to 1 (R^2 ranged from 0.9995 to 0.9998). As an example, in the Figure 3a it is depicted the above mentioned correlation performed for eucalyptol. Similarly, the absorbance values of PRHCl-thymol gel spectrum (200 values) were obtained as a linear combination of individual spectra of PRHCl gel and thymol gel (R^2 0.9988) (Figure 3b). A value of the correlation coefficient (R^2) close to 1, attests the lack of interaction between PRHCl and thymol in the respective gel. Consequently, these results attested the absence of a significant interaction between PRHCl and gel components.



a)



b)

Figure 3.

The correlation between absorbances from spectra of PRHCl hydroethanolic gels with eucalyptol/thymol or without terpenes

Determination of PRHCl content in hydroethanolic gels

The PRHCl content of HPMC-based hydroethanolic gels ranged from 97.85±0.63 to 102.47±0.54% of the theoretical value (3%, w/w), which complies with the pharmacopeial specifications for drug content. The obtained data indicated the uniform distribution of the drug within the hydroethanolic gels.

In vitro skin permeation study

In the present study the effect of five different terpenes namely eucalyptol (cyclic ether terpene), camphor (bicyclic ketone terpene), α-bisabolol (cyclic alcohol sesquiterpene), menthol (terpene alcohol) and thymol (terpene phenol) on the *in vitro* permeation of propranolol hydrochloride from HPMC-based hydroethanolic gels through pig ear skin was evaluated. The chemical structures of terpenes tested as enhancers in this study are given in Figure 1. The permeation profiles of PRHCl through pig ear skin in the absence or in the presence of 5% terpenes are depicted in Figure 4 and the corresponding values of permeation (steady state flux, J_{ss}, permeability coefficient, K_P and lag time, t_L) and release (release rate, k, diffusion coefficient, D) parameters are summarized in Table II.

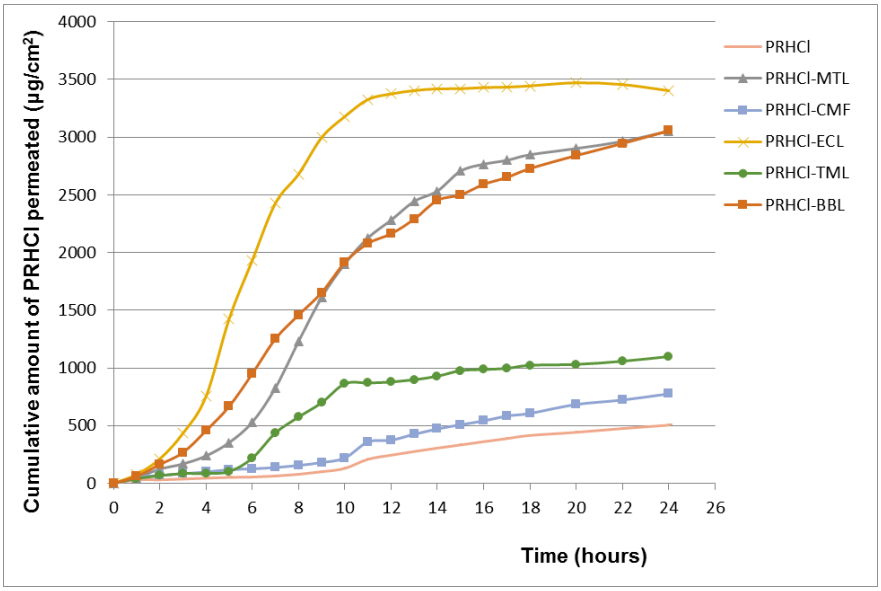


Figure 4.

In vitro propranolol hydrochloride permeation profiles through pig ear skin from HPMC-based hydroethanolic gels with or without terpenes as penetration enhancers (mean ± SD, n = 3)

Table II

The permeation and release parameters of the propranolol hydrochloride-loaded formulations through pig ear skin

Formulation code	Permeation parameters			Release parameters		ER_{flux}
	J_{ss} (μg/cm^2/h)	K_P x 10^{-6} (cm/h)	t_L (h)	k (μg/cm^2/h$^{1/2}$)	D x 10^{-5} (cm^2/h)	
PRHCl	35.19±2.41	11.73	5.55±0.91	239.35±2.06	4.996	1
PRHCl-MTL	316.08±3.56	105.36	4.12±1.65	1754.90±4.01	286.35	8.98
PRHCl-CMF	48.43±1.85	16.14	4.56±0.98	329.46±1.47	9.47	1.38
PRHCl-ECL	448.15±3.03	149.38	1.97±1.15	2139.10±3.25	399.11	12.74
PRHCl-TML	153.82±4.18	51.27	4.36±1.43	831.49±3.45	54.63	4.37
PRHCl-BBL	237.14±5.33	79.05	2.00±2.37	1268.30±4.63	140.30	6.74

These results indicate that the tested terpenes in this study can promote the propranolol hydrochloride transport through pig ear skin acting as enhancers. The most evident enhancing effect on the skin permeation of PRHCl was observed for eucalyptol, which provided the highest flux value, 12.7-fold greater than that obtained with the control gel. Also, menthol and α-bisabolol were effective enhancers for PRHCl, increasing the permeation rate 8.98- and 6.74-times than that of the control gel, respectively. It can be mentioned that for the first 9 h, PRHCl showed a better permeation from the formulation with α-bisabolol than that with menthol, but between 11 and 22 h the menthol containing gel showed slightly higher flux values compared to α-bisabolol gel. Significantly lower enhancing effect on the drug skin permeation presented thymol and camphor ($p < 0.05$), the last one being the less effective, because it provided a flux value only 1.38 fold higher than that of the control gel. These findings are consistent with previously reported propranolol hydrochloride permeation studies which indicated that 1,8-cineole (eucalyptol), menthol and α-bisabolol improved the skin permeation in a higher extent than other terpenes, i.e. camphor [2, 7]. The different effectiveness of the studied terpenes as percutaneous permeation enhancers of PRHCl could be attributed mainly to the variation in their interaction with the lipid components of the *stratum corneum*, variation related to several factors such as their structure and physicochemical properties (i.e. boiling point, partition coefficient between octanol and water, molecular weight). In another words, the enhancing activity of a terpene is the result of the combined effect of the above mentioned factors. The generally proposed mechanism for terpenes to promote the skin permeation of drugs is the increase in fluidity of the *stratum corneum* lipids by disrupting their intercellular packing. At the molecular level, oxygen-containing terpenes interact with polar heads of lipid bilayer forming

hydrogen bonds, simultaneously loosening the existent network of hydrogen bonds between ceramides [5, 25]. Consequently, the terpenes containing functional groups, which have the ability of hydrogen bonds formation, are effective permeation enhancers. Furthermore, a higher log P (partition coefficient between octanol and water) value of such compounds, reflecting a greater lipophilicity, is favorable because it facilitates the terpene association into the lipid bilayer of *stratum corneum*. Another physical property which might influence the potency of the terpene enhancers is the boiling point. A low boiling point indicates weak cohesiveness or self-association, thereby facilitating the terpene interaction with hydrogen bonds of *stratum corneum* lipids.

In the present study, eucalyptol (an ether terpene), menthol and α-bisabolol (monoterpene and sesquiterpene alcohols) were found to be the most effective enhancers for propranolol hydrochloride skin permeation. The aromatic alcohol terpene (thymol) showed moderate accelerant activity, whereas the ketone terpene (camphor) was a poor enhancer. Eucalyptol produced the highest skin permeation enhancement of PRHCl most probably due to the fact that it interacts with lipid components of *stratum corneum* more easily and extensively, because of its low boiling point (176-177°C) and hydroxyl group content. Of all the studied terpene, the boiling point of eucalyptol was approximately 29°C, 36°C, 56°C and 89°C lower than that of camphor (205°C), menthol (212°C), thymol (232.9°C), and α-bisabolol (265°C) respectively. In case of camphor, which contains a carbonyl group as hydrogen bond accepting moiety and has lower log P and boiling point values (2.089 and 205°C respectively) than the other terpenes, the interaction with the *stratum corneum* lipids by hydrogen bonding is weaker than that of other terpenes, because the structure and log P negative effect cannot be compensated by the favourable effect of boiling point on this interaction. These might explain the reason why camphor showed the lowest enhancing effect on the skin transport of PRHCl in the present study. Menthol was inferior in enhancing the skin permeation of PRHCl compared to eucalyptol, most probably due to its lower hydrogen-bonding ability determined by its higher boiling point value; in this case, the effect of terpene lipophilicity might be ignored as the log P values of menthol and eucalyptol are comparable (3.38 and 2.74 respectively). In case of thymol, it can be suggested that the extent of its interaction with the lipid bilayer by hydrogen bonding is less in comparison with menthol due to its higher boiling point, despite their similarity regarding the log P values (3.28 and 3.38 respectively) and the chemical class (cyclic monoterpene alcohols). This is the possible reason why the enhancing effect of thymol on PRHCl

skin permeation was lower than that of menthol. Among the alcohol terpenes, α-bisabolol was inferior in promoting the transdermal permeation of PRHCl compared to eucalyptol, but showed a similar activity as menthol. Cui et al. suggested that this sesquiterpene would interact easily with the lipid bilayer of the *stratum corneum*, because of the following properties: i) hydrogen bonding ability through the hydroxyl moiety existent in its structure; ii) high lipophilicity reflected by a log P value of 5.01, the highest among all the studied terpenes. These properties compensated the negative effect, induced by its strong cohesive or self-association (indicated by its boiling point value, 265°C, the highest among all the studied terpenes), on the interaction with the *stratum corneum* lipid components. Also, the above mentioned favourable characteristics might explain the superior enhancing effect of α-bisabolol on PRHCl skin permeation compared to thymol and camphor. This finding is consistent with the above mentioned study [7].

The presence of terpenes in the PRHCl gels decreased the lag time, another important parameter characterising the skin permeation process. From Table II, it can be observed that the PRHCl hydroethanolic gel without terpene (the control formulation) exhibited the maximum lag time value (5.55 h), while the formulations containing eucalyptol or α-bisabolol showed the shortest lag time (2 h). In the presence of menthol, thymol and camphor, PRHCl had a 1.3-fold lower lag time value than the control, with no significant differences between them, even the corresponding flux values were significantly different, decreasing in the following order: menthol > thymol > camphor (Table II). The differences between formulations in terms of lag time could be explained by the different contribution of each terpene in modulating the *stratum corneum* for higher permeability. In case of hydrophilic drugs, the extent of *stratum corneum* hydration is of great importance, influencing their partition coefficient into it. Also, it is well known that the hydration level of *stratum corneum* can be modified by the components of the vehicle. It was reported that ethanol at a concentration greater than 50 % v/v causes excessive dehydration of the skin [15]. In the present study, the co-solvent system selected for terpenes was 60% ethanol, which determined the skin dehydration, therefore PRHCl showed a long lag time in the control formulation. In the presence of terpenes, the lag time of PRHCl has decreased significantly ($p < 0.05$), especially in case of eucalyptol and α-bisabolol. This could be related to the disturbance of the *stratum corneum* integrity, thus facilitating the partition of drug into it and decreasing the time needed to reach the steady state of permeation. Furthermore, in the case of α-bisabolol, used as a humectant in cosmetics, the dehydration of *stratum corneum* is prevented; this can explain the

similarity between α-bisabolol and eucalyptol in reducing the PRHCl lag time.

The release rate (*k*) and diffusion coefficient (*D*) of drug from vehicle are also important factors influencing drug permeation as they could limit this process. In the present study, all studied terpenes significantly increased the PRHCl release rate and diffusion coefficient through vehicle as compared to those of the control ($p < 0.05$). Correlating the *in vitro* skin permeation results with the release results, the same ranking of PRHCl hydroethanolic gels was made based on both flux and release values: *PRHCl-ECL > PRHCl-MTL > PRHCl-BBL > PRHCl-TML > PRHCl-CMF > PRHCl.* It is to be mentioned that terpenes increase the PRHCl release parameters in a slightly lower extent than the permeation values. Furthermore, the release rate was greater than the transcutaneous flux in all cases, evidencing that the delivery of propranolol hydrochloride from hydroethanolic gels with or without terpenes through pig ear skin did not depend on the rate of its release from the formulations.

The results obtained from the *in vitro* PRHCl permeation of all hydroethanolic gel formulations through pig ear skin were kinetically evaluated by several mathematical models, namely zero-order, first-order, Higuchi and Korsmeyer-Pepas model. The results of curve fitting into the above mentioned mathematical models are presented in Table III and were evaluated by the highest correlation coefficient.

Table III

Results of kinetic analysis of the *in vitro* permeation data through pig ear skin obtained for propranolol hydrochloride-loaded formulations

Formulation code	Zero-order		First order		Higuchi		Korsmeyer-Pepas	
	$K_0(h^{-1})$	R^2	K_1 (h^{-1})	R^2	K_H (h^{-1})	R^2	*n*	R^2
PRHCl	0.4538	0.9538	0.0048	0.9532	1.4455	0.6902	0.9917	0.8029
PRHCl-MTL	3.0268	0.9127	0.0452	0.9392	11.25	0.7877	1.6289	0.9722
PRHCl-CMF	0.6623	0.9679	0.0071	0.9665	2.2598	0.7404	0.8711	0.177
PRHCl-ECL	3.0919	0.7439	0.0536	0.7897	15.6960	0.8383	1.477	0.9525
PRHCl-TML	1.051	0.8643	0.0118	0.8745	4.2291	0.8014	1.4093	0.8973
PRHCl-BBL	2.7665	0.937	0.0413	0.9719	11.247	0.8659	1.4392	0.9887

The comparison of the correlation coefficients obtained for each studied formulation after the curve fitting into various kinetic models (Table III), indicated that the zero-order model was the most suitable for describing the release kinetics of PRHCl from the control gel ($R^2 > 0.95$) and that with camphor ($R^2 > 0.96$), whereas the release of PRHCl from the gels with menthol, eucalyptol, thymol and α-bisabolol obeyed the Korsmeyer-Peppas

model (R^2 > 0.95, excepting *PRHCl-TML* with R^2 > 0.89) over a period of 24 hours. Moreover, in the case of the second group of formulations (fitting to Korsmeyer-Pepas model), the analysis of the first 60% of drug release data using the respective model was performed to determine the values of the diffusion exponent (n), an indicative of drug release mechanism: Fickian diffusion when $n \leq 0.5$, non-Fickian transport when $0.45 < n < 0.89$, case II transport when $n = 0.89$, and super case II transport when $n > 0.89$. According to the calculated values of the diffusion exponent, n, ranged between 1.4093 and 1.6289 (Table III), it can be suggested that the mechanism leading to the release of PRHCl from the respective systems was a super case II transport.

Conclusions

In the present study, HPMC-based hydroethanolic gels containing 3% propranolol hydrochloride and 5% terpene were developed in order to avoid the extensive hepatic first pass metabolism and achieve the desirable skin penetration rate of the drug. The results of our investigation indicated the effectiveness of alcohol terpenes in enhancing the skin transport of PRHCl most probably by fluidization of the *stratum corneum* lipid bilayer. Eucalyptol and α-bisabolol, which are different in terms of chemical structure and physicochemical properties (i.e. boiling point and log P), have the most pronounced enhancing activity on the skin permeation of PRHCl from HPMC-based hydroethanolic gels, which exhibited relatively shorter lag times and relatively higher permeation rates. *In vitro* release of PRHCl from the formulations containing eucalyptol and α-bisabolol in the presence of the skin revealed that this process was best described by the Korsmeyer-Pepas model. These hydroethanolic gels should be an alternative to oral dosage forms of PRHCl. However, further investigations regarding *in vitro* and *in vivo* studies on their stability, safety and therapeutic efficacy need to be performed in order to develop commercially viable topical formulation of propranolol hydrochloride.

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